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IN SILICO ANALYSIS OF SMALL MOLECULES FROM *SPIRULINA PLATENSIS* AS A LOOP DIERUTIC AND ANTI-INFLAMMATORY AGENT

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ABSTRACT

Spirulina platensis has been used as food for centuries by different populations and has only been rediscovered in recent years. Once classified as the - blue-green algae'', it does not strictly speaking belong to the algae, even though for convenience it continues to be referred to in that way. The current study aimed to explore the potential of *Spirulina platensis* extract as a loop diuretic and anti-inflammatory agent in silico. Gas Chromatography-Mass Spectrometry was used to identify the bioactive compounds in the *Spirulina platensis* extract. Seventeen bioactive compounds found from *Spirulina platensis* were then analyzed the loop diuretic-related activity using the Prediction of Activity Spectra for Substances (PASS) server. Furthermore, the SwissADME web server and ProTox II were used to evaluate drug-likeness, excretion, metabolism, distribution, absorption, and toxicity of the small molecule from *Spirulina platensis* based on PASS online prediction. There were 13 compounds found in *Spirulina platensis* that are related to loop diuretic and anti-inflammatory properties. Furthermore, several small molecules of *Spirulina platensis* (SP) have good bioavailability based on Swiss ADME analysis. Most of the Spirulina platensis small molecules followed the Lipinski rules and had high gastrointestinal (GI) absorption values. Based on these findings, small molecules of *Spirulina platensis* have the potential to act as loop diuretic and anti-inflammatory agents.

Keywords: Spirulina platensis, loop diuretic, anti-inflammatory, in silico.

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INTRODUCTION

Today, one in three adults suffers from high blood pressure, the leading cause of stroke and heart disease [1]. In addition, hypertension is also the cause of cardiac hypertrophy and heart failure (hypertensive heart disease), aortic rupture, and kidney failure. Changes in angiotensin I to II cause increased aldosterone secretion, sympathetic nerve activity, salt retention, and vasoconstriction of blood vessels so that blood pressure increases [2]. Several compounds that inhibit the action of the Angiotensin Converting Enzyme (ACE inhibitor) enzyme that has been produced synthetically include captopril, ramipril, and enalapril [3].

Indonesia, which is in the tropics has the potential for the development of microalgae and their derivative products. Recently, the polyphenols in Spirulina platensis have attracted attention because of their important role in health problems [4]. One that is very prospective is developing food products into nutraceutical products [5]. Nutraceutical is a substance that has physiological benefits or provides protection against chronic disease [6]. Grand View Research (2020) reports that the global nutraceutical market was valued at 382.51 billion USD in 2019 and is expected to continue to increase by 8.3% annually [7]. Currently, high blood pressure is one of the diseases that globally cause death [8]. Hypertension can also lead to cardiac hypertrophy, cardiovascular disease, aortic rupture, and renal failure [9]. Several studies have shown that risk reduction by various types of dietary polyphenols on hypertension and the main health risk factors that contribute to *cardiovascular disease* (CVD). The study showed that intake of polyphenol-rich foods, herbs, and beverages including flavonols, anthocyanidins, proanthocyanidins, flavones, flavanones, isoflavones, and flavan-3-ols, improves vascular health, thereby significantly reducing the risk of hypertension and cardiovascular disease [10]. Bioinformatics and in-silico analyses play a pivotal role in quickly designing, screening, and developing therapeutic drugs [11]. PASS has been a useful website for predicting biological spectral activity since 2000. Computer models of biological activity can predict biological activity in both published and new compounds, allowing for early screening of unpromising molecules. Computer-assisted simulations of drug excretion, metabolism, distribution, and absorption are being studied to apply an anticipatory and trustworthy complement of data to experimental accessions. The pharmacokinetic, physicochemical, and pharmacological characteristics of small compounds are predicted using this computational model [12]. SwissADME is a comprehensive and integrated website from the Swiss Institute of Bioinformatics (SIB), which offers bioinformatics recourses to researchers worldwide [13].

Therefore, this study aimed to explore the potential of Spirulina platensis as a loop diuretic and anti-inflammatory agent. This study uses computational analysis to evaluate and predict biological potential, drug-likeness, excretion, metabolism, distribution, absorption, and toxicity of *Spirulina platensis*.

MATERIALS AND METHODS

Spirulina Platensis Extraction Process

The extraction process begins by preparing a sample of 20 g of dry powder Spirulina platensis then dissolved in 100 ml of methanol solvent with a ratio between *Spirulina platensis* and methanol solvent of 1:5, using different concentrations of methanol solvent 50% until completely dissolved. The next step is the extraction process using a sonicator for 15 minutes with a frequency of 40 KHz. Then, the pure extract evaporated with a rotary evaporator until there was no more solvent dripping. The extraction results were obtained in the form of a thick extract.

Chemical Profile Screening

The experiment was carried out on an HP 6890 GCMS system (Hewlett-Packard, California, USA) with a capillary column (Agilent 19091S-433 HP-5MS; 30 m x 250 m i.d.; Santa-Clara, California, USA). The carrier gas was helium, with a 1 mL/min flow rate. The oven was preheated to 325°C. The pre-oven temperature was set to 150°C and maintained at a 2°C/min rate. It ran at 10°C/min for 10 minutes before increasing to 240°C for 11 min. Running took at least 24 min [14]. The scanning range was between 50 and 550 am. The quantification of the predicted compound was obtained from reading the area on the GC-MS graph. Estimation of the compound resulting from the GC-MS test was carried out using the Wiley/NIST Library software [15].

Computational Analysis

The initial step is to get canonical SMILE data from the PubChem server (https://pubchem.ncbi.nlm.nih.gov/) [16]. Then, SwissADME (http://www.swissadme.ch/) was used to estimate drug-likeness, excretion, metabolism, distribution, and absorption [17]. ProTox II (https://tox-new.charite.de/protox II/index.php?site=compound input) is used to determine toxicity [18]. The last step is using the PASS server (http://www.way2drug.com/PASSOnline/predict.php) to predict the biological activity of hydrolysate molecules by inputting canonical SMILE data [19].

RESULTS AND DISCUSSIONS

The small molecule profiling in the *Spirulina platensis* (SP) was analyzed using GC-MS. Based on GC-MS analysis, there were nine small molecules contained in *Spirulina platensis*. Table-1 showed that the major small molecule of *Spirulina platensis* was Pentadecanoic acid which was indicated by the highest percentage of the area (9.34%) with a retention time of 23.07 min. The other small molecules of Spirulina platensis C were 9-Octadecenoic acid (6.27 %), Hexadecanoic acid (5.17 %), and Hexadecanal (5.26 %). While the percentage of the area of other small molecules that were less than 5% were Heptadecanoic acid (2.08 %), 1-Nonadecene (2.38 %), 1,13-Tetradecadiene (2.20 %), 1-Hexacosene (2.17 %), 1,2-dimethyl-3-pentyl-4-propyl- Cyclohexane (2.19 %), 1,13-Tetradecadiene (2.20 %). The canonical SMILES and Pubchem ID can be seen in Table-2.

Table-1. Profile of small molecules from Spirulina platensis analyzed by GC-MS

Small molecule	Molecular formula	RT (Min)	Area (%)
Pentadecanoic acid	$C_{15}H_{30}O_2$	23.07	9.34
Octadecanoic acid	$C_{18}H_{36}O_2$	23.34	0.41
9-Octadecenoic acid	$C_{18}H_{34}O_2$	24.92	6.27
Hexadecanoic acid	$C_{16}H_{32}O_2$	25.36	5.17
Heptadecanoic acid	$C_{17}H_{34}O_2$	28.16	2.08
1-Nonadecene	$C_{19}H_{38}$	29.30	2.38
Oleic acid	$C_{18}H_{34}O_2$	29.93	1.25
Cyclotetracosane	$C_{24}H_{48}$	30.12	1.45
Cyclopentadecanone	$C_{15}H_{28}O$	30.49	1.46
Cyclohexane	C_6H_{12}	30.67	1.67
Hexadecanal	$C_6H_{12}O$	32.16	5.26
Z,E-3, 13-Octadecadien-1-ol	$C_{18}H_{34}O$	32.36	1.91
Z-2-Dodecenol	$C_{12}H_{24}O$	32.57	1.71
1-Hexacosene	$C_{26}H_{52}$	32.79	2.17
1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane	$C_{16}H_{32}$	33.33	2.19
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	33.75	1.62
1,13-Tetradecadiene	$C_{14}H_{26}$	33.85	2.20

Table-2. Pubchem ID and Canonical SMILES of small molecules from Spirulina platensis analyzed by GC-MS

Small molecule	Pubchem ID	Canonical SMILES)
Pentadecanoic acid	13849	CCCCCCCCCCCCCCC(=O)O
Octadecanoic acid	5281	CCCCCCCCCCCCCCCCCC(=O)O
9-Octadecenoic acid	637517	CCCCCCCCC=CCCCCCCCC(=O)O
Hexadecanoic acid	985	CCCCCCCCCCCCCCCC(=O)O
Heptadecanoic acid	10465	CCCCCCCCCCCCCCCCC(=O)O
1-Nonadecene	29075	CCCCCCCCCCCCCCCCCC=C
Oleic acid	445639	CCCCCCCCC=CCCCCCCCC(=O)O
Cyclotetracosane	520449	C1CCCCCCCCCCCCCCCCCCCCCCCC1
Cyclopentadecanone	10409	C1CCCCCCCC(=O)CCCCCC1
Cyclohexane	8078	C1CCCCC1
Hexadecanal	984	CCCCCCCCCCCCCCCC=O
Z,E-3, 13-Octadecadien-1-ol	5364516	CCCCC=CCCCCCCCCC=CCCO
Z-2-Dodecenol	5364955	CCCCCCCCCC=CCO
1-Hexacosene	29303	CCCCCCCCCCCCCCCCCCCCCCCCCC=C
1,2-dimethyl-3-pentyl-4-propyl- Cyclohexane	566238	CCCCCC1C(C(CCC1CCC)C)C
Bis(2-ethylhexyl) phthalate	8343	CCCCC(CC)COC(=O)C1=CC=CC=C1C(=O)OCC(CC)CCCC
1,13-Tetradecadiene	30875	C=CCCCCCCCCCCC=C

After identifying the small molecules of *Spirulina platensis*, the next step is to predict their biological activity using the PASS online program. Table-3 shows the prediction of biological potential as a loop diuretic using PASS online from small molecules of *Spirulina platensis*. Several groups of loop diuretic drugs include loop diuretics, anticholesterolemic, and anti-inflammatory such as pentadecanoic acid receptor antagonist (Pa 0.557), 9-octadecanoic acid receptor antagonist (Pa 0.744) and cyclotetracosane receptor antagonist (Pa 0.502). Other small molecules have an anti-inflammatory such as heptadecanoic acid with antagonist receptor Pa of 0.727.

Table-3. Small molecule from *Spirulina platensis* with Pa value higher than 0.4 using PASS online

Small molecule	PASS online		
	Pa	Pi	Activity
Pentadecanoic acid	0.557	0.005	Loop diuretic
Octadecanoic acid	0.557	0.005	Loop diuretic
9-Octadecenoic acid	0.744	0.006	Antihypercholesterolemic
Hexadecanoic acid	0.557	0.005	Loop diuretic
Heptadecanoic acid	0.727	0.002	Antiinflammatory, intestinal
1-Nonadecene	0.472	0.025	Antihypercholesterolemic
Oleic acid	0.744	0.006	Antihypercholesterolemic
Cyclotetracosane	0.502	0.007	Antiinflammatory
Cyclopentadecanone	0.649	0.003	Loop diuretic
Cyclohexane	0.649	0.003	Loop diuretic
Hexadecanal	0.496	0.003	Loop diuretic
Z,E-3, 13-Octadecadien-1-ol	0.498	0.022	Antihypercholesterolemic
Z-2-Dodecenol	0.469	0.010	Antiinflammatory, intestinal
1-Hexacosene	0.470	0.010	Antiinflammatory, intestinal
1,2-dimethyl-3-pentyl-4-propyl- Cyclohexane	0.517	0.052	Antiinflammatory
Bis(2-ethylhexyl) phthalate	0.537	0.046	Antiinflammatory
1,13-Tetradecadiene	0.427	0.017	Antiinflammatory

The pharmacokinetics parameters, bioavailability, and drug-likeness were also assessed (Tables 4 and 5). There are 6 out of 17 small molecules of *Spirulina platensis* that have good bioavailability. Bioavailability is an essential aspect to consider while developing nutraceuticals. The XLOGP3 (Table-6) of pentadecanoic acid and 1, 13-tetradecane were 6.63 and 7.35.

Table-4. Pharmacokinetics parameters and Bioavailability of the small molecule from *Spirulina platensis*

Small molecule	GI absorption	BBB permeant	Bioavailability score
Pentadecanoic acid	High	Yes	0.85
Octadecanoic acid	High	No	0.85
9-Octadecenoic acid	High	No	0.85
Hexadecanoic acid	High	Yes	0.85
Heptadecanoic acid	High	Yes	0.85
1-Nonadecene	Low	No	0.55
Oleic acid	High	No	0.85
Cyclotetracosane	Low	No	0.55
Cyclopentadecanone	High	Yes	0.55
Cyclohexane	Low	Yes	0.55
Hexadecanal	High	No	0.55
Z,E-3, 13-Octadecadien-1-ol	High	No	0.55
Z-2-Dodecenol	High	Yes	0.55
1-Hexacosene	Low	No	0.55
1,2-dimethyl-3-pentyl-4-propyl- Cyclohexane	Low	No	0.55
Bis(2-ethylhexyl) phthalate	High	No	0.55
1,13-Tetradecadiene	Low	No	0.55

Note: GI absorption - Gastrointestinal absorption; BBB permeant – blood-brain barrier permeation

Table-5. Druglikeness rule score of the small molecule from *Spirulina platensis*.

Small molecule	A	B	C	D	E
Pentadecanoic acid	Yes	Yes	No	Yes	No
Octadecanoic acid	Yes	No	No	No	No
9-Octadecenoic acid	Yes	No	No	No	No
Hexadecanoic acid	Yes	Yes	No	Yes	No
Heptadecanoic acid	Yes	No	No	No	No
1-Nonadecene	Yes	No	No	No	No
Oleic acid	Yes	No	No	No	No
Cyclotetracosane	Yes	No	Yes	No	No
Cyclopentadecanone	Yes	Yes	Yes	Yes	No
Cyclohexane	Yes	No	Yes	Yes	No
Hexadecanal	Yes	No	No	Yes	No
Z,E-3, 13-Octadecadien-1-ol	Yes	No	No	Yes	No
Z-2-Dodecenol	Yes	Yes	Yes	Yes	No
1-Hexacosene	Yes	No	No	No	No
1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane	Yes	No	Yes	Yes	No
Bis(2-ethylhexyl) phthalate	Yes	No	No	No	No
1,13-Tetradecadiene	Yes	Yes	No	Yes	No

Note:
A - molecular mass less than 500 Dalton;
B - high lipophilicity (expressed as LogP less than 5);
C - less than 5 hydrogen bond donors; D - less than 10 hydrogen bond acceptors;
E - molar refractivity should be between 40-130; F - conclusion.

The physicochemical properties of small molecules of *Spirulina platensis* can be seen in Table-7. These properties were used to determine their use in various processes, including chemistry, biology, and physics. The molecular weight and polarity of pentadecanoic acid was 242.40 g/mol and 37.30 Å2, respectively. The molecular weight and polarity of cyclohexane and hexadecenal are 84.16 and 240.42 g/mol with the polarity of 0.00 and 17.07 Å2. While the molecular weight and polarity of 1, 13-tetradecane were 194.36 g/mol and 0.00 Å2, respectively. Based on these results, the following compound has met the optimum criteria of its physicochemical properties.

Table-6. Characteristics of the small molecule from *Spirulina platensis.*

Small molecule	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log P	iLOGP
Pentadecanoic acid	3.66	6.63	5.16	3.94	4.81	4.84	3.66
Octadecanoic acid	4.30	8.23	6.33	4.67	6.13	5.93	4.30
9-Octadecenoic acid	4.27	7.64	6.11	4.57	5.95	5.71	4.27
Hexadecanoic acid	3.85	7.17	5.55	4.19	5.25	5.20	3.85
Heptadecanoic acid	4.11	7.69	5.94	4.44	5.69	5.57	4.11
1-Nonadecene	5.28	10.57	7.43	7.01	7.53	7.56	5.28
Oleic acid	4.27	7.64	6.11	4.57	5.95	5.71	4.27
Cyclotetracosane	4.97	10.60	9.36	7.85	6.74	7.91	4.97
Cyclopentadecanone	3.03	5.81	5.03	3.67	4.33	4.37	3.03
Cyclohexane	2.10	3.44	2.34	3.12	2.63	2.73	2.10
Hexadecenal	4.15	7.05	5.67	4.31	5.96	5.43	4.15
Z,E-3, 13-Octadecadien-1-ol	4.79	6.88	5.79	4.68	6.18	5.67	4.79
Z-2-Dodecenol	3.19	4.64	3.68	3.27	3.71	3.70	3.19
1-Hexacosene	6.89	14.37	10.16	8.51	10.63	10.11	6.89
1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane	4.07	7.51	5.67	6.04	4.84	5.63	4.07
Bis(2-ethylhexyl) phthalate	4.77	7.45	6.43	5.24	6.98	6.17	4.77
1,13-Tetradecadiene	3.95	7.35	5.26	4.77	5.31	5.33	3.95

Table-7. Physicochemical Properties of the small molecule from *Spirulina platensis*.

Small molecule	MW	HA	AHA	RB	HBA	HBD	MR	TPSA
Pentadecanoic acid	242.40	17	0	13	2	1	75.99	37.30
Octadecanoic acid	284.48	20	0	16	2	1	90.41	37.30
9-Octadecenoic acid	282.46	20	0	15	2	1	89.94	37.30
Hexadecanoic acid	256.42	18	0	14	2	1	80.80	37.30
Heptadecanoic acid	270.45	19	0	15	2	1	85.60	37.30
1-Nonadecene	266.51	19	0	16	0	0	92.97	0.00
Oleic acid	282.46	20	0	15	2	1	89.94	37.30
Cyclotetracosane	336.64	24	0	0	0	0	115.37	0.00
Cyclopentadecanone	224.38	16	0	0	1	0	72.30	17.07
Cyclohexane	84.16	6	0	0	0	0	28.84	0.00
Hexadecanal	240.42	17	0	14	1	0	79.23	17.07
Z,E-3, 13-Octadecadien-1-ol	266.46	19	0	14	1	1	88.85	20.23
Z-2-Dodecenol	184.32	13	0	9	1	1	60.49	20.23
1-Hexacosene	364.69	26	0	23	0	0	126.62	0.00
1,2-dimethyl-3-pentyl-4-propyl- Cyclohexane	224.43	16	0	6	0	0	76.91	0.00
Bis(2-ethylhexyl) phthalate	390.56	28	6	16	4	0	116.30	52.60
1,13-Tetradecadiene	194.36	14	0	11	0	0	68.46	0.00

Note:
MW - molecular weight (g mol-1); HA - number heavy atoms;
AHA - number aromatic heavy atoms; RB - number rotatable bonds; HBA - number hydrogen bond acceptor; HBD - number hydrogen bound donor; MR - molar refractivity (m3 mol-1); TPSA - topology polar surface area (Å²)

Based on Table-5, all molecules had a molecular mass of less than 500 Da, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and molar refractivity between 40-130. All small molecules from *Spirulina platensis* have a value between 1.17 and 4.12 on the D value, making them easy to make synthetically (Table-8). Table-9 shows oral toxicity prediction results from the small molecule of *Spirulina platensis*. 9-octadecanoic acid and oleic acid belong to class II (fatal if swallowed ($5 < LD50 \leq 50$)) and were based on ProTox II. Mutagenicity and cytotoxicity prediction results from all small molecules of *Spirulina platensis* were negative.

Table-8. Medicinal chemistry prediction of the small molecule from *Spirulina platensis*

Small molecule	A	B	C	D
Pentadecanoic acid	No; 3 violations; MW<250	0	0	2.20
Octadecanoic acid	No; 2 violations: Rotors>7, XLOGP3>3.5	0	0	2.54
9-Octadecenoic acid	No; 2 violations: Rotors>7, XLOGP3>3.5	0	1	3.07
Hexadecanoic acid	No; 2 violations: Rotors>7, XLOGP3>3.5	0	0	2.31
Heptadecanoic acid	No; 2 violations: Rotors>7, XLOGP3>3.5	0	0	2.42
1-Nonadecene	No; 2 violations: Rotors>7, XLOGP3>3.5	0	1	2.93
Oleic acid	No; 2 violations: Rotors>7, XLOGP3>3.5	0	1	3.07
Cyclotetracosane	No; 1 violation: XLOGP3>3.5	0	0	3.57
Cyclopentadecanone	No; 2 violations: MW<250, XLOGP3>3.5	0	0	2.53
Cyclohexane	No; 1 violation: MW<250	0	0	1.17
Hexadecanal	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	0	1	2.26
Z,E-3, 13-Octadecadien-1-ol	No; 2 violations: Rotors>7, XLOGP3>3.5	0	1	3.43
Z-2-Dodecenol	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	0	1	2.69
1-Hexacosene	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	0	1	3.77
1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane	No; 2 violations: MW<250, XLOGP3>3.5	0	0	3.52
Bis(2-ethylhexyl) phthalate	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	0	1	4.12
1,13-Tetradecadiene	No; 3 violations: MW<250, Rotors>7, XLOGP3>3.5	0	1	2.31

Note:
A - Leadlikeness; B -Pan Assay Interference Structures;
C - Structural Alert; D - Synthetic accessibility score;

Table-9. Oral toxicity prediction results from the small molecule of *Spirulina platensis* using ProTox II

Small molecule (Class)	LD50	HE	CA	IM	MU	CY
Pentadecanoic acid (4)	900	(-)	(-)	(-)	(-)	(-)
Octadecanoic acid (4)	900	(-)	(-)	(-)	(-)	(-)
9-Octadecenoic acid (2)	48	(-)	(+)	(-)	(-)	(-)
Hexadecanoic acid (4)	900	(-)	(-)	(-)	(-)	(-)
Heptadecanoic acid (4)	900	(-)	(-)	(-)	(-)	(-)
1-Nonadecene (6)	5050	(-)	(-)	(-)	(-)	(-)
Oleic acid (2)	48	(-)	(-)	(-)	(-)	(-)
Cyclotetracosane (3)	750	(-)	(+)	(-)	(-)	(-)
Cyclopentadecanone (5)	5000	(-)	(-)	(-)	(-)	(-)
Cyclohexane (3)	178	(-)	(+)	(-)	(-)	(-)
Hexadecanal (5)	5000	(-)	(-)	(-)	(-)	(-)
Z,E-3, 13-Octadecadien-1-ol (4)	1016	(-)	(+)	(-)	(-)	(-)
Z-2-Dodecenol (5)	5000	(-)	(+)	(-)	(-)	(-)
1-Hexacosene (6)	5050	(-)	(-)	(-)	(-)	(-)
1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane (6)	15380	(-)	(-)	(-)	(-)	(-)
Bis(2-ethylhexyl) phthalate (4)	1340	(-)	(+)	(-)	(-)	(-)
1,13-Tetradecadiene (6)	5050	(-)	(+)	(-)	(-)	(-)

Note:
LD50-Lethal Dose 50% of response (mg kg-1); HE - hepatotoxicity; CA - carcinogenicity; IM - immunotoxicity; MU - mutagenicity; CY - cytotoxicity; (-) - inactive; (+) - active; class 1 - fatal if swallowed ($LD50 \leq 5$); class 2 - fatal if swallowed ($5 < LD50 \leq 50$); class 3 - toxic if swallowed ($50 < LD50 \leq 300$); class 4 - harmful if swallowed ($300 < LD50 \leq 2000$); class 5 - may be harmful if swallowed ($2000 < LD50 \leq 5000$); class 6 - non-toxic ($LD50 > 5000$).

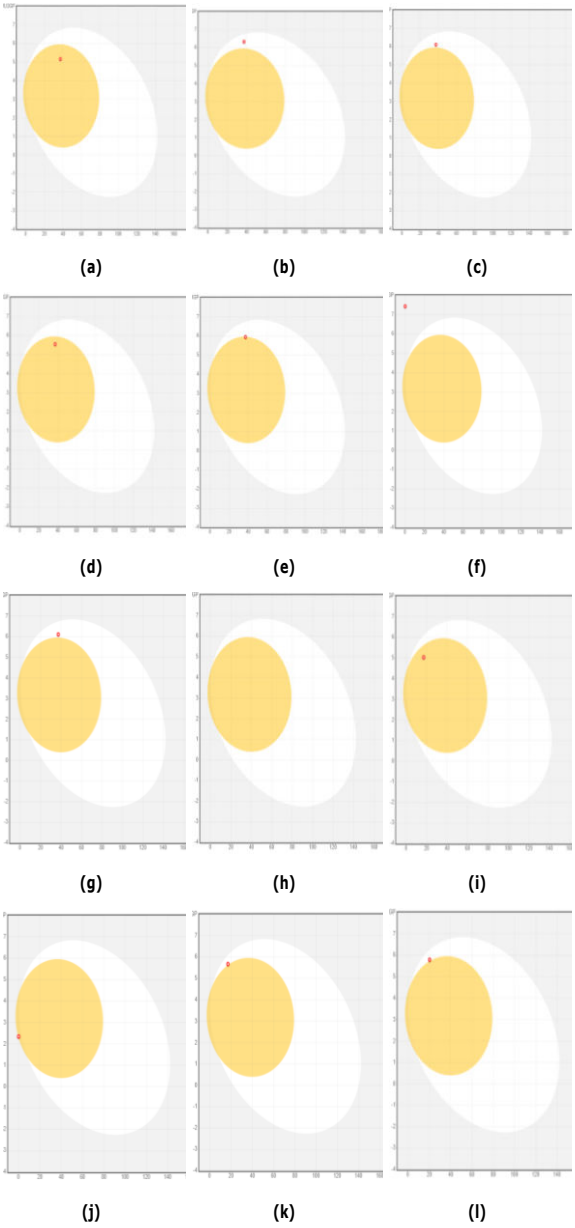

Figure-1. BOILED-Egg diagram for Drug-likeness of the small molecule from *Spirulina platensis*: (a) Pentadecanoic acid (b) Octadecanoic acid (c) 9-Octadecenoic acid (d) Hexadecanoic acid (e) Heptadecanoic acid (f) 1-Nonadecene (g) Oleic acid (h) Cyclotetracosane (i) Cyclopentadecanone (j) Cyclohexane (k) Hexadecanal (l) Z,E-3, 13-Octadecadien-1-ol (m) Z-2-Dodecenol (n) 1-Hexacosene (o) 1,2-dimethyl-3-pentyl-4-propyl-Cyclohexane (p) Bis(2-ethylhexyl) phthalate (q) 1,13-Tetradecadiene

Pentadecanoic acid and cyclohexane have the potential activity as loop diuretic and anti-inflammatory respectively. If the Pa value is more than 0.7, the compounds in the experiment are active. On the other hand, the chemical may resemble other known drug agents. The PASS online could predict over 300 pharmacological properties and biochemical pathways [20]. The PASS online can be used more accurately to detect novel targets or agents for specific ligands. The PASS online tool effectively discovers new ligands for specific protein targets [21]. The program introduces PASS Online, a free online resource. It intends to accurately predict the biological activity spectra of organic compounds based on their structural formula for over 4,000 different categories of biological properties. The prediction is analyzed according to structure-activity interactions in the experimental dataset, containing information on over 300,000 organic compounds [22].

The molecular and physicochemical activity such as molecular weight, molecular formula, H-bond donors, H-bond acceptors, rotatable bonds, heavy aromatic atoms, heavy atoms, molar refractivity, and TPSA are among the physicochemical properties of *Spirulina platensis* small molecules (Table-3). The findings were calculated using Open Babel v 2.3.0 [23]. The PSA is computed by applying the polar atoms sulfur and phosphorus and a fragmental technique known as TPSA or topological polar surface area [24]. The *Spirulina platensis* small molecules general features revealed that the molecular weight of all compounds was less than 500 g/mol and then indicated as a key attribute that can be referred to as drug-likeness.

The characteristics of the *Spirulina platensis* small molecules are shown in Table-6. SwissADME offers five free models for determining a compound's lipophilicity character: XLOGP3, WLOGP, MLOGP, SILICOS-IT, and iLOGP. XLOGP3, a knowledge-based library and an atomistic approach with corrective features [25]. WLOGP is a purely atomistic method for dealing with a fragmented system [10]. MLOGP is a topological approach archetype that employs 13 molecular descriptors and a linear relationship [26]. SILICOS-IT is a hybrid approach that employs 7 topological descriptors and 27 fragments. In iLOGP, solvation-free energies in n-octanol and water are determined using the generalized-born and solvent-accessible surface area (GB/SA) model. The log P o/w is the mean of the values projected from 5 suggested approaches [27].

The GI absorption of all *Spirulina platensis* small molecules drugs was quite high, except 1-nonadecene, cyclotetracosane, cyclohexane, 1-hexacosene, 1,2-dimethyl-3-pentyl-4-propyl-cyclohexane, 1,13-tetradecadiene that indicating low absorption (Table 4). This suggests that the majority of the *Spirulina platensis* small molecules have good absorption. The Egan egg is an elliptical region populated by well-absorbed molecules that were utilized to test the model's efficacy for GI passive absorption and prediction of brain access via passive diffusion to lay the BOILED-Egg (Brain or Intestinal L Estimate D permeation predictive model). The Lipinski rule is the first of 5 guidelines for identifying tiny compounds according to physicochemical parameter profiles such as NH or OH $\leq$ 5, molecular weight less than 500, N or O $\leq$ 10, and MLOGP $\leq$ 4.15 [28]. According to Lipisnki's rules, all small molecules from *Spirulina platensis* matched the drug-likeness criteria (Table-5).

Based on Figure-9, it can be seen that there are red dots outside the EGG, inside the white BOILED-Egg, and the yellow EGG. The red dot indicates the digestibility

of specific compounds of *Spirulina platensis* that have been extracted. Correlation bioavailability and egg figure. The red dot outside the BOILED-Egg indicates that the compounds anlayzed have no digestibility in the human body, which means it has low GI absorption and bioavailability. For example, Figure 9 (f) 1-nonadecene with low GI absorption and 0.55 bioavailability has a red dot outside the EGG. The red dot inside the white BOILED-Egg indicates the compound has a good digestability and it is delivered through the body but not including the brain cell. For example, Figure 9 (c) 9-octadecenoic acid with high GI absorption and 0.85 bioavailability has a red dot inside the white BOILED-Egg. This indicates that 9-octadecenoic acid has good digestibility through the human body but the compound is not yet delivered to human brain cells. The red dot inside the yellow EGG indicates the compound has a good digestability and it is delivered through the body including the brain. For example, Figure 9 (a) pentadecanoic acid with high GI absorption and 0.85 bioavailability has a red dot inside the yellow BOILED-Egg. This indicates that pentadecanoic acid has good digestibility through the human body and the compound is delivered to human brain cells.

The Lilly MedChem [7] rule used to purify chemical libraries of compounds that are likely to be unstable, reactive, toxic, or prone to interfere with biological assays due to their structure has been based on the root of structural alert [6], the pan assay interference compounds or PAINS structural alert [4], or the root of structural alert [6]; [29]. The synthetic accessibility (SA) score is predicated on the concept that the frequency of molecular fragments in 'really' available compounds relates to synthesis ease. Ertl and Schuffenhauer [30] reported the created and validated method as being characterized by the molecular synthetic accessibility value, which varied from 1 to 10 (easy to very difficult). Drug-induced hepatotoxicity is a primary cause of abrupt liver failure and one of the leading causes of drug rejections [5]; [31]. Drug-induced liver injury (DILI) might be long-term or occur only once. The ProTox-II hepatotoxicity prediction model has an equitable reliability of 82.00 % on cross-validation and an accuracy of 86.00 % on external validation [32]; [33].

CONCLUSIONS

The current study suggested that small molecules from the small molecules of *Spirulina platensis* may have potential as loop diuretic and anti-inflammatory agents. 11 out of 17 small molecules met the pharmacokinetic criteria. Further research should be done in vitro and in vivo to elucidate the small molecules from *Spirulina platensis*, which might be used as a loop diuretic agent and further develop excellent nutraceutical products.

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REFERENCES

[1] Statistics Indonesia. 2020. Ekspor dan impor [Export and Import]. https://www.bps.go.id/exim/

[2] Statistics Indonesia. 2021. Ekspor dan impor [Export and Import]. https://www.bps.go.id/exim/

[3] Ali J., Camilleri P., Brown M. B., Hutt A. J., Kirton,S. B. 2012. Revisiting the general solubility equation: in silico prediction of aqueous solubility incorporating the effect of topographical polar surface area. Journal of Chemical Information and Modeling 52:420-428.

[4] Baell J. B., Holloway G. A. 2010. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and their exclusion in bioassays. Journal of Medicinal Chemistry 53:2719-2740.

[5] Banerjee P., Eckert A. O., Schrey A. K., Preissner R. 2018. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Research 46:W257-W263.

[6] Brenk R., Schipani A., James D., Krasowski A., Gilbert I. H., Frearson J., Wyatt P. G. 2008. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. ChemMedChem. 3: 435-444.

[7] Bruns R. F., Watson, I. A. 2012. Rules for identifying potentially reactive or promiscuous compounds. Journal of Medicinal Chemistry. 55: 9763-9772.

[8] Mills K. T., Stefanescu A. and He, J. 2020. The global epidemiology of hypertension. Nature Reviews Nephrology, 16(4): 223-237. https://doi.org/10.1038/s41581-019-0244-2

[9] Mensah G. A. 2016. Hypertension and target organ damage: don't believe everything you think!. Ethnicity & Disease, 26(3): 275-278. https://doi.org/10.18865/ed.26.3.275.

[10] Chandran A., Merlin N. J., Ammu L., Dharan S. S. 2019. Fennel Treatment to PCOS: An Insilico Evaluation to explore the Therapeutic Efficacy of Anethole. Research Journal of Pharmacy and Technology. 12: 4958-4962.

[11] Zloh M., Kirton S. B., 2018. The benefits of in silico modeling to identify possible small-molecule drugs and their off-target interactions. Future Medicinal Chemistry. 10: 423-432.

[12] Sliwoski G., Kothiwale S., Meiler J., Lowe E. W. 2014. Computational methods in drug discovery. Pharmacological Reviews. 66: 334-395.

[13] Ndombera F., Maiyoh G. and Tuei V. 2019. Pharmacokinetic, Physicochemical and Medicinal Properties of N-Glycoside Page 2 of 8 Anti-Cancer Agent more Potent than 2-Deoxy-D-Glucose in Lung Cancer Cells. Journal ofPharmacy and Pharmacology, 6(1): 1-8. https://doi.org/10.17265/2328-2150/2019.04.003

[14] Riyadi P. H., Tanod W. A., Sulistiyati D. T., Aulanni'am A. A., Suprayitno E. 2021. Effects of nile tilapia (Oreochromis niloticus) viscera hydrolysate on blood pressure, TNF-α and IL-6 expression in rats (Rattus norvegicus) induced by DOCA-salt. Indian Journal of Animal Research. 55: 1-6.

[15] Tanod W. A., Dewanto D. K., Ndobe S., Riyadi P. H., Putra M. Y. 2019. Screening of antibacterial and antioxidant activity of soft corals Sinularia sp. and Sarcophyton sp. from Palu Bay Central Sulawesi. Squalen: Bulletin of Marine and Fisheries Postharvest and Biotechnology 14:73–83.

[16] Kim S., Thiessen P. A., Bolton E. E., Chen J., Fu G., Gindulyte A., Han L., He, J., He S., Shoemaker B. A., Wang J., Yu B., Zhang J. and Bryant S.H. 2016. PubChem substance and compound databases. Nucleic Acids Research, 44(D1), D1202-D1213. https://doi.org/10.1093/nar/gkv951

[17] Christina Y. I., Nafisah W., Atho'illah M. F., Rifa'i M., Widodo N., Djati M. S., 2021. Anti-breast cancer potential activity of Phaleria macrocarpa (Scheff.) Boerl. leaf extract through in silico studies. Journal of Pharmacy & Pharmacognosy Research 9:824-845.

[18] Riyadi P. H., Atho'illah M. F., Tanod W. A., Rahmawati I. S. 2020. Tilapia viscera hydrolysate extract alleviates oxidative stress and renal damage in deoxycorticosterone acetate-salt-induced hypertension rats. Veterinary World. 13: 2477-2483.

[19] Riyadi P. H., Darmanto Y. S., Anggo A. D., Sumardianto S., Rianingsih L. 2020. Color test for screening chemical components of protein hydrolyzed extract from non-shell small crab (Portunus Pelagicus) Waste. International Journal Science and Technology Research. 9: 6-9.

[20] Lagunin A., Stepanchikova A., Filimonov D. and Poroikov V. 2000. PASS: prediction of activity spectra for biologically active substances. Bioinformatics, 16(8): 747-748. https://doi.org/10.1093/bioinformatics/16.8.747

[21] Dai S. X., Li W. X., Han F. F., Guo Y. C., Zheng J. J., Liu J. Q., Wang Q., Gao Y. D., Li G. H. and Huang J. F. 2016. In silico identification of anti-cancer compounds and plants from traditional Chinese medicine database. Scientific Reports, 6(1): 1-11. https://doi.org/10.1038/srep25462

[22] Filimonov D. A., Lagunin A. A., Gloriozova T. A., Rudik A. V., Druzhilovskii D. S., Pogodin P. V. and Poroikov V. V. 2014. Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds, 50(3): 444-457. https://doi.org/10.1007/s10593-014-1496-1

[23] O'Boyle N. M., Banck M., James C. A., Morley C., Vandermeersch T. and Hutchison G. R. 2011. Open Babel: An open chemical toolbox. Journal of Cheminformatics, 3(1): 1-14. https://doi.org/10.1186/1758-2946-3-33

[24] Ertl P., Rohde B. and Selzer P. 2000. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. Journal of Medicinal Chemistry, 43(20): 3714-3717. https://doi.org/ 10.1021/jm000942e

[25] Tripathi P., Ghosh S., Talapatra S. N. 2019 Bioavailability prediction of phytochemicals present in Calotropis procera (Aiton) R. Br. by using Swiss-ADME tool. World Scientific News. 131: 147-163.

[26] Eros D., Kövesdi I., Orfi L., Takács-Novák K., Acsády G. and Kéri, G. 2002. Reliability of logP predictions based on calculated molecular descriptors: a critical review. Current Medicinal Chemistry, 9(20): 1819-1829. https://doi.org/10.2174/0929867023369042

[27] Daina A., Michielin O. and Zoete V. 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1): 1-13. https://doi.org/10.1038/srep42717

[28] Raschka S., Wolf A. J., Bemister-Buffington J. and Kuhn L. A. 2018. Protein-ligand interfaces are polarized: discovery of a strong trend for intermolecular hydrogen bonds to favor donors on the protein side with implications for predicting and designing ligand complexes. Journal of Computer-aided Molecular Design, 32(4): 511-528. https://doi.org/10.1007/s10822-018-0105-2

[29] Irwin J. J., Duan D., Torosyan H., Doak A. K., Ziebart K. T., Sterling T., Tumanian G. and Shoichet, B. K.. 2015. An aggregation advisor for ligand discovery. Journal of Medicinal Chemistry. 58(17): 7076-7087. https://doi.org/10.1021/acs.jmedchem.5b01105

[30] Ertl P. and Schuffenhauer A. 2009. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. Journal of Cheminformatics, 1(1): 1-11. https://doi.org/10.1186/1758-2946-1-8

[31] Siramshetty V. B., Nickel J., Omieczynski C., Gohlke B. O., Drwal M. N., Preissner R. 2016. WITHDRAWN-a resource for withdrawn and discontinued drugs. Nucleic Acids Research 44:D1080-D1086.

[32] Chen M., Suzuki A., Thakkar S., Yu K., Hu C., Tong W. 2016 DILIrank: the largest reference drug list ranked by the risk for developing drug-induced liver injury in humans. Drug Discovery Today. 21:648-653.

[33] Riyadi, P. H., Sari, I. D., Kurniasih, R. A., Agustini, T. W., Swastawati, F., Herawati, V. E. and Tanod, W. A., 2021, October. SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in spirulina platensis. In IOP Conference Series: Earth and Environmental Science (Vol. 890, No. 1, p. 012021). IOP Publishing. https://doi.org/10.1088/1755-1315/890/1/012021.